INTERNAL REPORT

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June 2009

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Summary

- Around Tasmania, the benthic macrofauna of marine-influenced embayments and large estuaries exhibited a strong regional separation between Macquarie Harbour and the eastern and northern coasts, with the Macquarie Harbour fauna depauperate in species and animal numbers.

- Introduced species comprised a dominant presence in terms of biomass across much of Tasmania, but were present in negligible densities in Macquarie Harbour.

- The scale of fish farm impacts was substantially less than the scale of natural regional variation.

- The inshore Tasmanian marine environment was undergoing a period of change, with increasing organic loadings to sediments, declining sediment redox potential, and increasing modal particle size at sites investigated over the period of study.

- Associated with these environmental changes was an increasing density of macrofauna, which included an increasing proportional abundance of both introduced taxa and capitellid polychaetes.

- Fish farm effects that extended to regional scales and affected reference sites could not be adequately assessed within the project because reference regions without fish farms were not monitored; however, significant increases through time at reference and compliance sites in sediment organic matter, surface redox potential, total macrofaunal abundance, proportional abundance of capitellid polychaetes, and proportional abundance of introduced species, were are all suggestive that organic enrichment associated with fish farms may have extended at low levels across regional scales.

- Given the implications to biodiversity conservation of region-wide impacts, monitoring of reference sites in regions lacking fish farms is urgently warranted.

- Fish farm effects were found to significantly affect the sediment environment and macrobenthic communities near farm leases in all major regions of the state other than Macquarie Harbour, where no effect of fish farm activity was detected.

- Fish farm effects were most apparent in comparisons between reference sites and farm sites.

- Fish farm effects were also detectable at compliance sites located 35 m out from lease boundaries, where sediment redox potential and faunal assemblage composition were intermediate between patterns found at farm and reference sites; however, farm effects were relatively minor at compliance sites with no indication of negative impacts on the biota.

- The major physical effect of fish farm activity was a decline in redox potential of sediments to at least 4 cm depth.

- The faunal community within fish farms generally exhibited increased faunal dominance, increased proportional abundance of capitellids, and decreased total bivalve/total mollusc ratio.

- Species found to be consistently positively-associated with fish farm impacts included the capitellid polychaete *Capitella* sp., the leptostracan crustacean *Nebalia* sp., the nereid polychaete *Neanthes cricognatha*, the ostracod crustacean *Euphilomedes* sp., the introduced bivalve *Theora lubrica*, and the nassarid gastropod *Nassarius nigellus*.

- Some species were also found to be negatively-associated with fish farm impacts, but such species tended to be localised in distribution and not generate significant results in statewide analyses.

- Introduced species were increasing in proportional abundance within the macrofauna by 2-3% per annum.

- The most abundant and widespread introduced species were the bivalves *Theora lubrica* and *Corbula gibba*, and the screwshell *Maoricolpus roseus*, while the bivalve *Raeta pulchella* and fanworm *Euchone limnicola* were also locally abundant.

- Future taxonomic study of taxa identified during the project to the genus level will likely reveal additional introduced species that are abundant within the state but not currently recognised as introduced.

- Populations of *M. roseus* were stable through time and unaffected or slightly negatively-affected by fish farm activity.

- Populations of *C. gibba* appear newly established within Tasmania, with exponential growth between 1998 and 2001, and a subsequent population decline. This bivalve was positively associated with sites with high organic loading and fine sediments, but was not strongly associated with fish farms.

- Populations of *T. lubrica* were positively affected by fish farm activity, increasing through time at farm and compliance sites but not at reference sites.

- Increasing population numbers of introduced species were generally associated with increasing population numbers of native species, rather than the introduced taxa causing a detectable decline in native populations through competition.

- The Tasmanian finfish monitoring program comprises a successful partnership between industry, management and researchers that has provided an invaluable state-wide baseline for assessing environmental impacts within estuarine and inshore marine habitats. Given the magnitude of ecological changes evident over the six-year period of study, we strongly recommend that this monitoring scheme be continued through the long-term and expanded to include regions that currently lack fish farms.

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1. Introduction

Salmonid production using open-water marine cages has intensified exponentially worldwide during the past two decades. Although a number of environmental impacts associated with salmonid aquaculture are now recognised (including export and translocation of disease and parasites, genetic dilution of native stocks, adverse effects on benthos, altered nutrient cycling, and deoxygenation, Black, 2001), research at the global level on the environmental consequences of salmon culture has failed to keep track with growth in the fishery. Little published research is available describing field investigations of the environmental impacts of salmonid culture for many of the major growing regions of the world. Most such investigations focus on impacts on macrobenthic invertebrates, a community type amenable to research and one that can undergo extreme change when subjected to high levels of organic enrichment associated with farm operations (Hargrave *et al.*1997; Karakassis *et al.* 1999, 2000; Macleod *et al.* 2004, 2006; Pereira *et al.* 2004; Pohle *et al.* 2001; Ritz *et al.*1989). Unfortunately, the spatial scale of published investigations rarely extends beyond one or two fish farm lease areas; hence, given the magnitude of regional variation, few generalities associated with environmental impacts are currently recognised, and opinions on the scale of impacts vary widely.

In Tasmania, government environmental regulations associated with fishfarm operations stipulate “no unacceptable visual, chemical or biological impact on the benthos 35 m beyond the boundary of the marine farming lease area. Relevant environmental parameters must be monitored in the lease area, 35 m from the boundary of the marine farm lease area and at any control site(s)” (Crawford, 2003). To comply with these regulations, routine monitoring of the benthic environment using systematic methods was undertaken in the vicinity of all Tasmanian salmonid farms between 1997 and 2003 (Woods *et al.* 2004).

During the 6-year period from 1997, habitat quality at replicated sites located within farms, and 35 m from farm boundaries, was monitored at 6 monthly intervals by video, and benthic invertebrates and sediments were sampled at 2-year intervals using cores and grabs at replicated sites located within farms and 35 m from farm boundaries, plus control reference locations. We here synthesise results from the invertebrate and sediment sampling component of this long-term program, which arguably represented world’s ‘best-practice’ for a government-mandated environmental monitoring scheme of fish farms. Since 2003, the scale of the Tasmanian finfish monitoring scheme has been reduced following the review by Woods *et al.* (2004). All new farms are still required to undertake a baseline survey; however, existing farms are now only required to undertake annual qualitative video surveys. In the event that the video surveys reveals unacceptable visual impacts then licence conditions allow for a triggered quantitative physio-chemical and biological survey to be required.

Our study follows an initial investigation of broadscale effects of salmonid aquaculture in southeastern Tasmania (Edgar *et al*. 2005). The present study expands on the earlier study by including analysis of temporal change to more rigorously identify environment impacts, and by encompassing all salmonid growing regions around the Tasmanian coastline. Given the prevalence of introduced marine pests in Tasmanian waters, and the possibility that such pests interact with fish farm operations, the data set has also be used to assess impacts of introduced species on the benthic environment during the period of study.

2. Methods

2.1 Sampling protocols

Environmental and biological data were obtained in collaboration with, and in some cases by, salmonid farm operators from 42 separate farm lease locations distributed in six regions around Tasmania (Fig. 1). Most farm leases were located in southeastern Tasmania and Macquarie Harbour, with outlying leases on the north and east coasts. Sampling primarily occurred from 1998 to 2003, although two farm leases were sampled in 1997 and a single lease in 2004. Farm leases varied in size and production capacity but were typically 20-60 ha, with 6-20 cages (26-36 m diameter) stocked per lease. Cages were periodically moved within farm lease areas, with rotation times of 12-36 months. Depths of sites studied ranged from 4 m to 47 m, with a mean of 20 m.

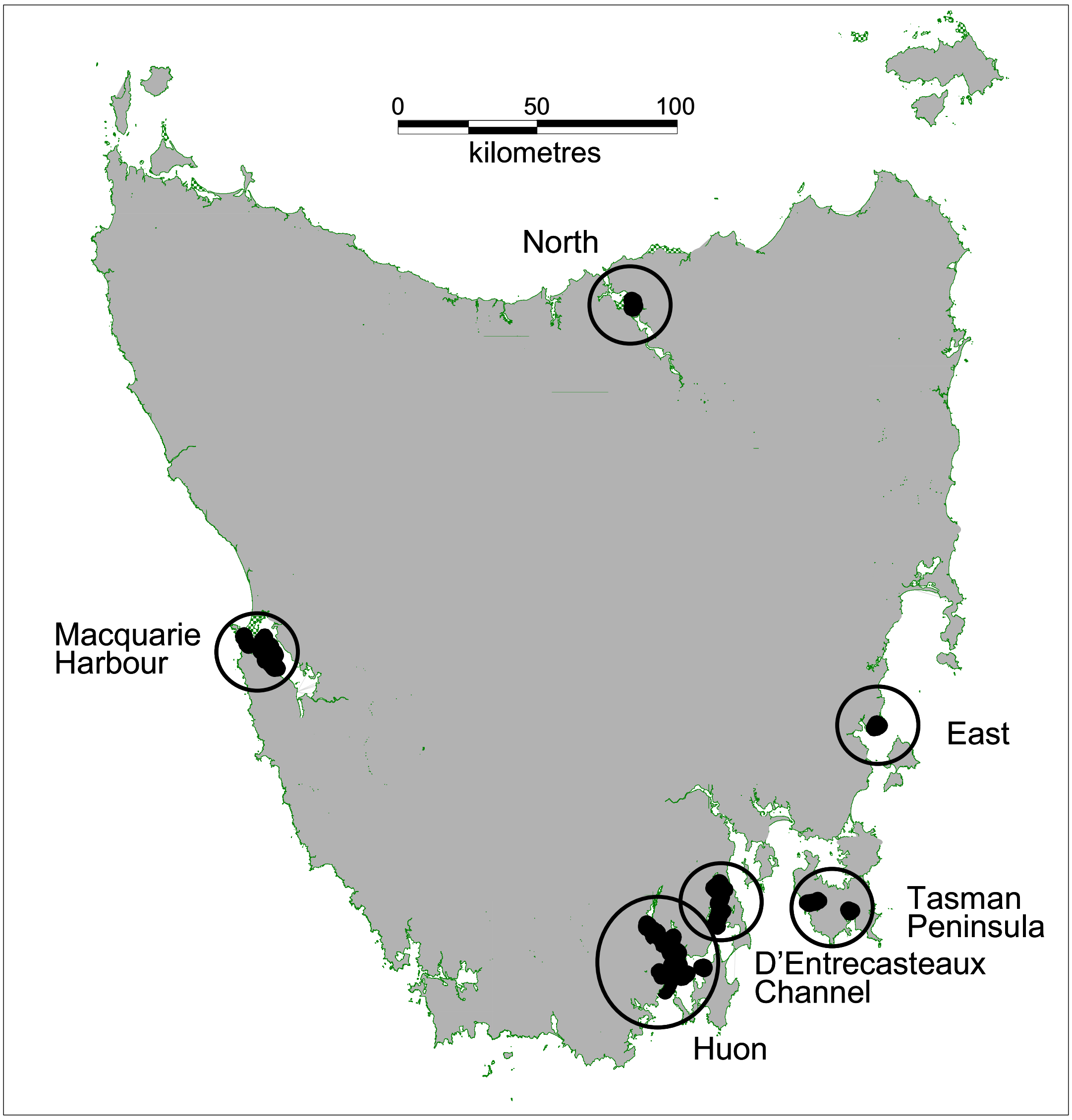

Figure 1. Regions of Tasmania with salmonid fish farm leases.

Sites sampled within each farm lease were located by differential GPS and categorised by level of farm impact as: (i) 'farm' lease sites, located within the lease area, (ii) 'compliance' sites, located 35 m outside the lease boundary, and (iii) control 'reference' sites, located at distance (generally 1-2 km) from the lease boundary in a similar depth to the associated lease. Many farm leases were first surveyed prior to commencement of farming, with these baseline data considered to also represent 'reference' conditions. A total of 411 sites were sampled during the study, with each site sampled an average of 1.5 times.

Sampling location and frequency reflected government conditions associated with environmental monitoring of each lease area. Mandatory monitoring requirements stipulated by the Tasmanian Department of Primary Industries, Water and Environment varied between leases, depending on size and shape of lease. Most lessees were obliged to monitor on a two-yearly cycle: (i) one reference site, (ii) four compliance monitoring sites distributed at 35 m distance off each edge of the four sides of the lease area, and (iii) two farm sites within the lease area. Lessees were also obliged to undertake baseline surveys prior to commencement of farming at 4 to 18 sites, the number of sites varying with lease area. Individual sites sampled during baseline surveys, including reference sites, were relocated using differential GPS and resampled on subsequent biennial monitoring occasions wherever possible; however, lease boundaries and monitoring requirements changed through time, precluding resampling of sites in many instances.

In accordance with government requirements, triplicate macrofaunal samples were collected <5 m apart at each site using either a Van Veen grab (0.07 m^2 surface area) or diver operated corer (150 mm diameter) pushed into the sediment to a depth of 100 mm. Samples were collected by four organisations (Aquenal Pty Ltd., AMD Pty Ltd, Aquatas Pty Ltd and Nortas Pty Ltd), with the great majority (92%) of samples collected using grab by one organisation (Aquenal Pty Ltd).

Samples were sieved in the field using either a 1 mm-mesh sieve or by placing sample material into 1 mm-mesh bags. Material retained after sieving was transferred into vials and fixed with 5% buffered formalin. In the laboratory, the sample was washed through a stacked series of sieves (1, 1.4, 2, 2.8 and 4 mm) using the methods described by Edgar (1990). Material retained on each sieve was sorted, with animals separated into species groups and counted. Identification of specimens in all samples was undertaken by AD and GJE using a reference collection to provide consistency in species attribution of undescribed taxa.

A Craib corer was used alongside the Van Veen grab at each site to collect triplicate sediment cores (43 mm diameter) for analysis of sediment properties. Redox potential was measured in millivolts at the sediment surface and at 40 mm depth in the sediment. The standard potential of the Ag/AgCl reference cell of the probe was 199 mV. Calibration and functionality of the meter were checked before each test using a redox buffer solution (220 mV at 25 °C). Measurements were made within 3 hours of sample collection.

After redox measurements were completed, two subsamples were separated from each Craib core for analysis of particle size and organic content, as described in more detail by Edgar *et al.* (2005). Organic content was calculated for the top 30 mm of sediment as the percentage loss of dry mass on ignition at 450°C. Particle size distribution was assessed volumetrically by filling a measuring cylinder with sample material, then washing material through a stack of sieves (4 mm, 2 mm, 1 mm, 500 µm, 250 µm, 125 µm and 63 µm). The content of each sieve was drained and transferred to the measuring cylinder, commencing with the coarsest sediment fraction and working through to the finest. The cumulative volume of sediment in the measuring cylinder was recorded after the content of each sieve was transferred. The fraction <63 µm diameter was calculated by difference from the level of the original sample in the measuring cylinder.

2.2 Univariate analyses

The effects of fish farming activity on the sediment environment and macrobenthos were assessed using F-tests associated with Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA; Zar, 1996), as calculated by SYSTAT (Wilkinson, 1987). In order to avoid pseudoreplication (Hurlbert, 1984) resulting from differences in the number of replicate sites sampled per farm lease, data were aggregated as the mean value for each of the three levels of impact (farm, compliance and reference) at each farm lease. Although the precision of these estimates of the true mean will vary with the number of sites sampled within the farm lease, such differences in precision should not greatly affect outcomes of statistical tests.

Regional variation in the effects of fish farm activity was assessed using two-way ANOVA, where sites within the major fish farm regions (Huon, D'Entrecasteaux Channel, Tasman Peninsula and Macquarie Harbour) were grouped. To maintain a symmetric statistical design, data from lease areas lacking information for all three effect types were excluded from analysis, as were data from the north region, where data were only available for two farm leases. Lease replication was unbalanced with 10, 8, 5 and 4 leases investigated in the Huon, D'Entrecasteaux Channel, Tasman Peninsula and Macquarie Harbour regions, respectively. Null hypotheses tested were that no differences existed between sites grouped into the three impact levels across the range of farm leases, nor between regions. Farm effect and region were both regarded as fixed factors.

Response variables assessed as potential indicators of farm activity included five sediment and seven macrofaunal metrics (Table 1). These metrics included the most important indicators of farm activity identified by Edgar *et al*. (2005). Data for these metrics were not transformed for ANOVA because little heteroscedasticity was detected using boxplots (see Fig. 3).

Table 1. Metrics examined as potential indicators of farm activity.

1. Median particle size (phi-scale)
2. Silt-clay content (particles <63 µm; %)
3. Redox potential at sediment surface
4. Redox potential at 40 mm depth
5. Organic matter (%), measured as loss on ignition
6. Total number of species per replicate sample
7. Total number of individuals per replicate sample
8. Dominance calculated as percentage contribution of species with highest abundance
9. Evenness calculated using inverse Simpsons Index[1]
10. Introduced taxa as percentage of total faunal abundance
11. Capitellid polychaetes as percentage of total faunal abundance
12. Bivalves as percentage of total mollusc abundance

[1] $=1/\Sigma(n_i/N)^2$ where n_i is density of *i*th species and N is total sample abundance

An additional set of ANOVAs were undertaken using the same potential univariate indicators of farm activity, but with analyses based on temporal data. These one-way ANOVAs utilised data from the subset of sites that were initially sampled as reference localities and later re-surveyed, with each data point calculated as the difference per site between the mean value of a metric at the end of a two-year monitoring cycle and the mean value at the start. To accommodate the biennial monitoring cycles, data obtained during each two-year period were aggregated for analyses (1998-99, 2000-01, 2002-03; with data from the two leases studied in late 1997—Brabazon Point and Brabazon Point—included with data for 1998-99).

The same analytical process involving one-way ANOVA and temporal data calculated as the difference in abundance between start and end of each two year monitoring cycle was used to assess the significance of relationships between densities of common animal species and fish farm effects. Boxplots describing variances in animal densities were often heterogeneous and highly skewed; consequently animal density data analysed by ANOVA were transformed using a fourth root transformation, the same transformation as used in multivariate analyses.

Interpretation of ANOVA results requires caution, given the large number of tests undertaken and probability of associated Type I statistical errors. Nevertheless, following Anderson, *et al.* (2008), we did not adjust probability levels using Bonferoni or other correction because of the likelihood that a large number of Type II errors would thereby be introduced, where no significant change was detected despite a real change. The number of Type II errors introduced using an adjusted probability level would have greatly exceeded the number of Type I errors avoided.

Analysis of Covariance (ANCOVA) was utilised to assess whether densities of introduced species differed significantly between the three biennial sampling periods (1998-99, 2000-01, 2002-03), or were affected by farm effects. Year was modelled as a continuous linear variable and farm effect as a fixed categorical factor in these analyses, with farm lease included in models as a blocking factor.

2.3 Multivariate analyses

Faunal relationships between sites were investigated using non-metric multidimensional scaling (MDS) plots calculated by PRIMER (Plymouth, U.K; Clarke and Warwick, 1994). These provided the best graphical depictions in two dimensions of biological similarities between sites.

As in univariate analyses, sample data were aggregated as a mean value for the three levels of farm impact for each farm lease. Rare species contributed little to multivariate patterns and were excluded from multivariate analyses if observed at <5% of sites. ‘Species x site’ data matrices showing mean abundances of different invertebrate species at different sites were initially fourth root transformed, then converted to matrices of biotic similarity between pairs of sites using the Bray-Curtis similarity index, as recommended by Faith *et al.* (1987). The fourth root transformation greatly reduces the relative influence of the most abundant species in analyses, but was considered appropriate here because some sites were dominated by individual species, which would otherwise largely exclude other species at those sites from contributing to observed multivariate pattern. The usefulness of the two dimensional MDS display of biotic relationships is indicated by the stress statistic, which signifies a good depiction of relationships when <0.1 and poor depiction when >0.2 (Clarke, 1993).

The SIMPER module of PRIMER was used to identify species that contributed most substantially to the average similarity within each biogeographic region (Clarke, 1993), and thus could be considered to typify each region. SIMPER was also used to identify species strongly associated with farm, compliance and reference sites.

Another module of PRIMER, ANOSIM, was applied to the same matrices to assess whether faunal assemblages differed significantly between sites subjected to different levels of farm impact. In order to minimise regional variation, lease area was included as a blocking factor and two-way ANOSIMs used, with the factor of primary interest ‘farm effect’.

Canonical Analysis of Principal Co-ordinates (CAP) was used to identify faunal relationships with environmental metrics (Anderson, 2003). CAP is a constrained ordination procedure that initially calculates unconstrained principal coordinate axes, followed by canonical discriminant analysis on the principal coordinates to maximise separation between sites related to environmental metrics. CAP is considered more flexible than direct canonical discriminant analysis because any dissimilarity measure can be utilised rather than only Mahalanobis distance (Anderson and Robinson, 2003; Anderson and Willis, 2003). The same similarity matrix was used for this analysis as in MDS, SIMPER and ANOSIM, with triplicate sample data aggregated as a mean value for each site, site data then aggregated as a mean value for each treatment at each farm lease, and fourth root transformation. Pearson correlation coefficients were calculated between environmental metrics and each of the first five CAP axes to identify which metric had strongest biological association.

The CAP procedure was also used to assess farm effects, including calculation of probability values associated with differences between farm, compliance and reference groupings of sites. Probability values were quantified as miscalculation error using the ‘leave-one-out’ approach. Each data point was removed from the data set, the CAP analysis rerun using the remaining observations, and then the removed data point classified to the nearest group centroid in canonical space. Comparison of known with allocated groups provided the miscalculation error (Anderson and Willis, 2003).

3. Results

3.1 Faunal composition and regional distribution

A total of 109,260 animals was collected during the benthic monitoring program from amongst 1833 samples, comprising 1001 taxa. The richest and most numerous of the major taxa were crustaceans, polychaetes, bivalves and gastropods, with 439, 288, 98 and 92 species, and 38,754, 35,402, 21,879 and 7625 individuals collected, respectively (Table 2).

Table 2. Number of individuals and number of species collected for each major taxonomic group.

Taxon	Number	Species
Anemone	409	9
Platyhelminth	15	7
Nemertean	1035	9
Oligochaete	4	2
Polychaete	35402	288
Leech	1	1
Crustacean	38754	439
Insect	1	1
Pycnogonid	9	3
Sipunculan	103	4
Phoronid	228	1
Brachiopod	1	1
Aplacophoran mollusc	33	1
Bivalve mollusc	21879	98
Amphineuran mollusc	10	4
Gastropod mollusc	7625	92
Squid	1	1
Echinoderm	3786	32
Enteropneust	30	2
Fish	14	6

The most widespread species collected around Tasmania was the nassarid gastropod *Nassarius nigellus*, while three bivalve species were also widely collected (Table 3). The introduced bivalve species *Corbula gibba* and *Theora lubricata* were the most abundant and the third most abundant species collected, respectively. The second most abundant taxon was the pollution indicator *Capitella* sp., a polychaete that is possibly also introduced. The taxon *Capitella* sp. may include more than one morphologically-indistinguishable species.

Table 3. Abundance and occurrence of species recorded at >100 sites or with >800 sampled individuals. Species recorded at the same site on two sampling occasions are regarded for tabulation purposes here as occurring at two sites.

Species	Number	Sites	Taxon
Nassarius nigellus	2731	276	Mollusc gastropod
Corbula gibba	8831	248	Mollusc bivalve
Ennucula obliqua	2039	238	Mollusc bivalve
Theora lubrica	3887	236	Mollusc bivalve
Euphilomedes sp.	3734	222	Crustacean
Callianassa limosa	1238	215	Crustacean
Amphiura elandiformis	1700	210	Echinoderm
Echinocardium cordatum	793	200	Echinoderm
Kalliapseudes sp.	2003	191	Crustacean
Lumbrinereis sp.	2212	190	Polychaete
Terebellides sp.	800	178	Polychaete
Ostracod sp.	1319	176	Crustacean
Maoricolpus roseus	1878	174	Mollusc gastropod
Chaetozone setosa	513	158	Polychaete
Nemertean sp.	391	150	Nemertean
Sthenelais pettibonae	297	150	Polychaete
Lysilla jennacubinae	1004	149	Polychaete
Paraprionospio coora	954	146	Polychaete
Asychis sp.	980	144	Polychaete
Nemocardium thetidis	1149	140	Mollusc bivalve
Amphicteis sp.	1294	133	Polychaete
Edwardsia sp.	282	132	Anemone
Ampelisca cf. australis	3164	131	Crustacean
Leitoscoloplos bifurcatus	677	128	Polychaete
Dimorphostylis cottoni	441	123	Crustacean
Nemertean sp.	382	121	Nemertean
?Ungulinid sp.	420	119	Mollusc bivalve
Heteromastus sp.	1914	113	Polychaete
Byblis mildura	3106	109	Crustacean
Hexapus granuliferus	165	106	Crustacean
Euchone limnicola	596	103	Polychaete
Processa sp.	223	102	Crustacean
Capitella sp.	7165	61	Polychaete
Sphaeromatid sp.	3024	24	Crustacean
Apseudid sp.	2106	40	Crustacean
Nephtys australiensis	988	92	Polychaete
Ampharetid sp.	954	71	Polychaete
Cacozeliana granarium	951	21	Mollusc gastropod
Parawaldeckia stebbingi	894	49	Crustacean
Polydora sp.	880	51	Polychaete
Tipimegus thalerus	803	98	Crustacean

Regional patterns of faunal similarity between lease areas are shown as an MDS plot in Fig. 2, which is based on the most widespread 204 species (ie., those that occurred in 18 or more sets of 3 triplicate grabs). Farm leases with close faunistic relationships group closely together in this figure, while farm leases plotted at distance have few species in common. Sample data used to calculate this plot were aggregated as a mean value for different farm impact treatments at each lease, and fourth root transformed to reduce the influence of abundant species.

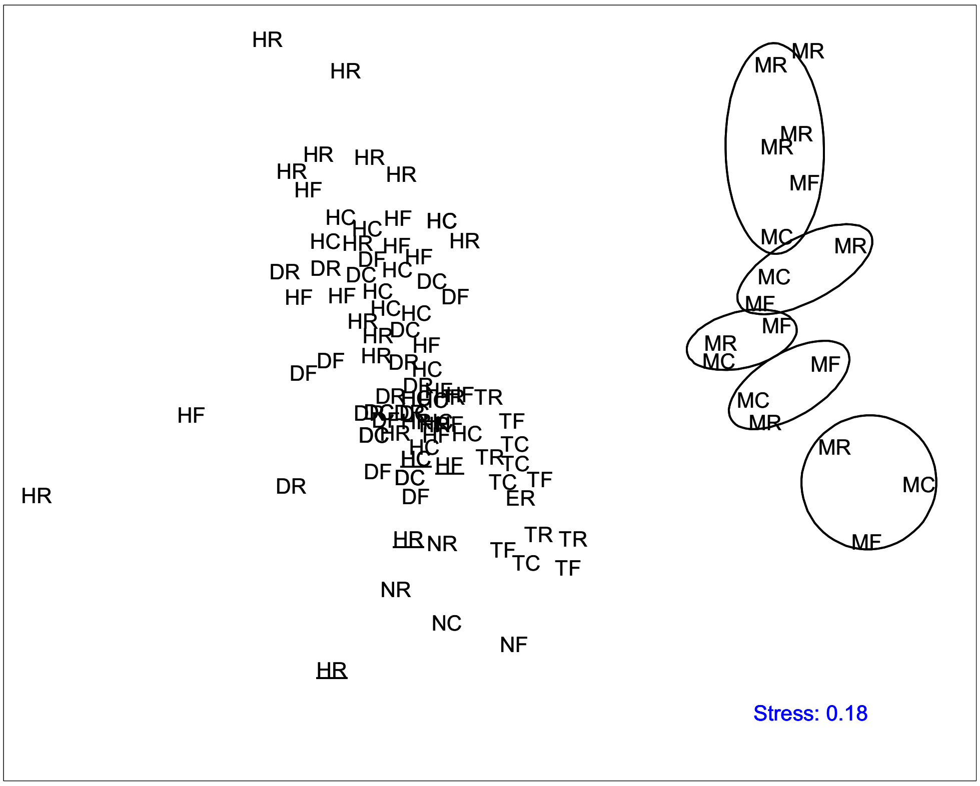

Figure 2. MDS depiction of relationships between benthic faunas sampled at farms in different regions of Tasmania (H: Huon; D: D'Entrecasteaux Channel; T: Tasman Peninsula; E: East coast; N: North coast; M: Macquarie Harbour) at three levels of farm impact (R: reference; C: compliance monitoring; F: farm). The first letter in each symbol refers to region, the second to farm impact level. To provide an indication of variation at different levels of farm impact within a lease, the three impact levels associated with five separate farm leases at Macquarie Harbour are encircled.

The major faunal disjunction observed was between sites sampled in Macquarie Harbour and those sampled elsewhere around the Tasmanian coast. Tasman Peninsula leases grouped closely together in the lower centre of the figure, with the east coast lease at Okehampton also positioned amongst this group. Because of close geographic and biological similarities between these regions, the east coast lease has been included within the Tasman Peninsula region in analyses described below.

Lease areas on the eastern side of the southern D'Entrecasteaux Channel (Satellite Island and Zuidpool) were initially categorised with leases in the Huon region, but show greater affinity with northern D'Entrecasteaux Channel sites. These leases are marked with an initial 'H' and symbols underlined in Fig. 2, and are located in the lower centre of the figure. Because of closer faunistic affinity to D'Entrecasteaux Channel leases, these leases are included within the latter region in analyses described below. North coast sites in the Tamar River also show a moderate faunal affinity with this group.

Variation associated with farm activity was low in comparison with variation between leases within a region, as indicated by the relatively small area encompassed by reference, compliance and farm sites at individual leases in Fig. 2, where the three treatments from individual leases at Macquarie Harbour are encircled. Although not shown in Fig. 2 for reasons of graphical clarity, faunas associated with different farm treatments within leases in other regions were of comparable magnitude. While reference sites generally trend above farm and compliance sites in Fig. 2, the three treatments are interspersed across the plot rather than concentrated together, as would be evident if substantive farm effects were occurring.

The indicator species most characteristic of each region were identified using SIMPER analysis (Table 4). The D'Entrecasteaux Channel and the Huon had many indicator species in common, whereas indicator species in Macquarie Harbour were generally poorly represented elsewhere. The best indicator of the D'Entrecasteaux Channel environment, the introduced bivalve *Corbula gibba*, was also the primary indicator in the Huon. The second best D'Entrecasteaux Channel indicator, another introduced bivalve (*Theora lubrica*), was also an important species in the Huon and Tasman Peninsula regions.

Table 4. Mean densities per site sampled of regional indicator species. Indicator species were identified by SIMPER analysis as the species that best characterise each region, with rank in importance for the 12 most important species within each region shown as superscript.

Species	D'Entrecasteaux Channel	Huon	Macquarie Harbour	North	Tasman Peninsula
Corbula gibba	43.76[1]	21.15[1]	0	0	0.43
Theora lubrica	17.29[2]	6.77[5]	0	9.50[3]	1.21
Lumbrineris sp.	12.73[3]	0.61	0.03	0.05	1.05
Callianassa limosa	2.98[4]	3.66[4]	0	0	0.67
Ostracod sp.	5.99[5]	1.93[10]	0	1.98	0.41
Ennucula obliqua	7.16[6]	4.52[7]	0	0.14	0.27
Kalliapseudes sp.	8.48[7]	1.43	0	0	1.02
Lysilla jennacubinae	3.99[8]	1.46	0.05	5.77	0.38
Amphiura elandiformis	4.77[9]	5.14[3]	0	0	0.41
Nassarius nigellus	5.05[10]	7.38[2]	0.01	1.93	5.98[1]
Nemocardium thetidis	1.95[11]	3.34	0	0	1.09
Paraprionospio coora	1.52[12]	2.99	0.01	0	0.11
Asychis sp.	1.17	1.74[11]	0	0	6.43
Sthenelais pettibonae	0.21	1.19[6]	0.08	0.02	0.16
Terebellides sp.	1.24	2.26[8]	0	0.82	1.66
Edwardsia sp.	0.19	0.75[9]	0.42[12]	0.38[12]	0.55
Euphilomedes sp.	6.65	2.28[12]	5.39[3]	0.07	19.15[7]
Leitoscoloplos bifurcatus	1.21	0.17	4.88[1]	5.63	0.74
Thyasira sp.	0	0	5.76[2]	0	0
Echinocardium cordatum	0.45	1.06	0.63[4]	0	3.74[2]
Chaetozone setosa	0.71	0.83	0.96[5]	0	1.24
Terebellid sp.	0.06	0	0.76[6]	0	0.53
Pista australis	0.63	0.05	2.11[7]	0.02	5.69
Oedicerotid sp.	0.22	0.17	0.67[8]	0.04	0.58
Ampharetid sp.	0	0	0.58[9]	0	0
Sabellid sp.	0	0	0.31[10]	0	0
?Ungulinid sp.	0.63	0.40	0.89[11]	0	0
Branchiomma sp.	0.11	0	0	35.13[1]	0.03
Nephtys australiensis	2.60	0.02	0.02	8.56[2]	6.59
Amphiura sp.	0.01	0.04	0.01	7.89[4]	0
Orbinia sp.	0.26	0.24	0	5.48[5]	0.06
Melitid sp.	0.42	0.09	0	17.13[6]	0.01
Lumbrinereis sp.	0.02	0	0	4.59[7]	0.41
Harmothoe sp.	0.43	0.26	0	1.63[8]	0.18
Halicarcinus ovata	0.05	0.03	0	0.93[9]	0.86[11]
Armandia sp.	0.09	0.12	0	0.78[10]	0.54
Xenocheira sp.	1.21	0	0	1.86[11]	0
Amphicteis sp.	0.52	2.60	0	0.52	6.87[3]
Heteromastus sp.	0.81	0.62	0.74	5.64	15.43[4]
Maoricolpus roseus	2.91	2.66	0.03	0.41	9.68[5]
Halicarcinus rostratus	0.59	0.07	0	0.25	2.13[6]
Ophiura kinbergi	0.02	0.06	0.01	0	5.45[8]
Ampelisca cf. australis	2.61	3.80	0	0	20.65[9]
Dimorphostylis cottoni	0.20	0.64	0	0.13	3.01[10]
Cyclaspis caprella	0.04	0.06	0	0	4.12[12]

The physical and geographical factors with strongest influence on benthic community structure were identified using CAP analysis, which maximises faunal differences between sites in canonical space in relation to a set of physical metrics. This analysis was based on the same similarity matrix as used to derive the MDS plot of Fig. 2.

Site easting showed an extremely high correlation with the first CAP axis (Table 5), indicating that geographic separation of sites towards the east and west of Tasmania influenced benthic faunal composition much more than other factors. This separation reflected a major difference between the depauperate fauna at Macquarie Harbour and faunas sampled elsewhere around the Tasmanian coast. The second CAP axis was strongly correlated with percentage organic matter, silt-clay content, and modal particle size, indicating that aspects of sediment type possessed the second strongest influence on benthic faunal composition. Other variates were not strongly correlated with CAP axes, although moderate correlations were evident between CAP axis 3 and northing, CAP axis 4 and redox potential at 4 cm depth, and CAP axis 5 and redox potential at the sediment surface. Unexpectedly, depth appeared to have little influence on faunal composition within the range of sites examined.

Table 5. Correlations between CAP canonical axes and nine geographical and physical variates.

Variate	Axis				
	1	2	3	4	5
Easting	-0.91	0.20	0.17	0.033	-0.02
Northing	0.53	-0.47	0.39	-0.26	0.07
Depth	-0.34	0.31	-0.18	-0.27	-0.22
Phi	0.57	0.62	0.08	0.18	-0.13
Silt	0.54	0.66	0.20	-0.13	-0.11
Redox 0 cm	-0.22	0.18	-0.15	-0.20	0.42
Redox 1 cm	-0.24	0.15	-0.18	-0.22	0.46
Redox 4 cm	-0.21	0.25	-0.06	-0.37	0.32
% Organic matter	0.39	0.70	0.19	0.13	-0.01

3.2 Spatial effects- univariate metrics of fish farming impacts

The significance of fish farm effects on the sediment environment was assessed using two-way ANOVA based on the mean across all times for samples in reference, compliance and farm locations within each of the different lease areas. To maintain a symmetric statistical design, data from lease areas lacking information for all three effect types were excluded from analysis, as were data from the north coast region, where little data were available.

All 12 potential indicators of farm activity exhibited significant variation between regions (Table 6). Five metrics (surface redox, redox at 4 cm depth, faunal dominance, faunal evenness, and bivalve/mollusc ratio) varied significantly between farm impact levels, and this farming effect was consistent across different regions. Farm effect also influenced the proportion of capitellids in samples; however, the magnitude of this effect varied between regions. This interaction primarily resulted from little increase in numbers of capitellids in farm lease areas in the Macquarie Harbour region, compared to an order of magnitude increase at farms elsewhere (Table 7).

Table 6. Mean-square values (MS) and F-ratios resulting from two-way ANOVAs (fixed factors: farm effect and region) for 12 metrics of farming activity. ***, P<0.001; **, 0.001<P<0.01; * 0.01<P<0.05.

Metrics	Farm effect		Region		Farm effect x Region		Error
	MS	F	MS	F	MS	F	MS
Particle size	0.20	0.16	13.74	11.31***	0.25	0.20	1.22
Silt-clay	437	0.79	5091	9.20***	229	0.41	553
Redox 0 cm	71300	9.64***	31300	4.24**	4300	0.58	7400
Redox 4 cm	18100	6.46**	27500	9.92***	2200	0.80	2800
Organic matter	7.4	0.21	432.2	12.53***	12.9	0.37	34.5
Total species	19	0.13	2368	15.73***	8	0.06	151
Total abundance	53000	3.29	229300	14.26***	14600	0.91	16100
Dominance	0.08	4.00*	0.05	2.35*	0.01	0.50	0.02
Simpson's Index	35.9	2.54	43.4	3.07*	3.7	0.26	14.1
Introduced species	15	0.05	4082	13.66***	19	0.06	299
Capitellids	827.8	10.57***	374.5	4.78**	168.3	2.15*	78.3
Bivalves/molluscs	3590	9.70***	4630	12.46***	320	0.85	370

Table 7. Mean percentage of capitellids amongst total fauna for samples collected in different regions.

Region	Reference	Farm	Monitoring
D'Entrecasteaux Channel	1.61	20.93	4.30
Huon	0.61	8.70	1.49
Macquarie Harbour	0.79	1.64	0.69
Tasman Peninsula	1.79	26.76	9.26
All regions	1.06	13.77	3.18

Differences in mean values associated with the three levels of farm activity are presented in Fig. 3 for each of the 12 metrics examined. Because characteristics of samples varied greatly between farm lease locations, a large sample variance is associated with each plot, somewhat obscuring farm effects. For the metrics found by ANOVA to show significant farm effects: (i) mean redox levels and the bivalve/mollusc ratio at reference sites were higher than at compliance sites, which were higher than at farm sites, (ii) mean faunal dominance was higher at farm sites than at reference and compliance sites, which were similar, (iii) the proportion of capitellids was lower at reference than at compliance sites, which was lower than for farm sites. Standard error bars at farm sites were relatively high for redox and proportion of capitellids, indicating considerable patchiness in values for these metrics at farm sites.

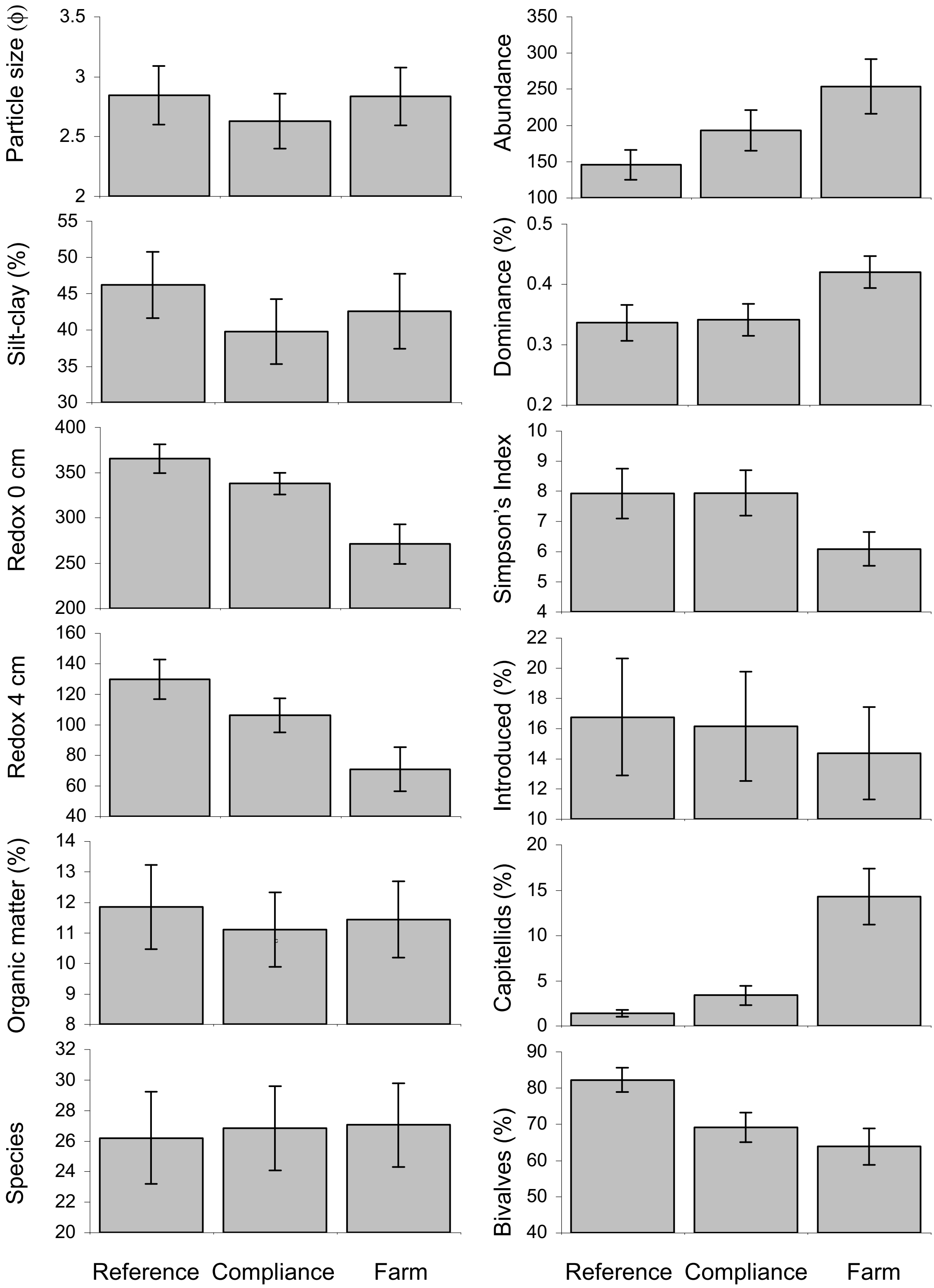

Figure 3. Mean (± standard error) for 12 metrics associated with the sediment environment and fish farm activity.

3.3 Spatial effects- multivariate indicators of fish farming impacts

As with univariate analyses, the effect of fish farm activities on faunal community structure was initially analysed by calculating the mean across all times for samples in reference, compliance and farm locations at each of the different lease areas. Data from lease areas lacking information for all three effect types were excluded from analyses to maintain a balanced statistical design. Following this data reduction, density information for the 204 most widespread species (ie those that occurred at 18 or more sites) from 28 lease areas were analysed using two-way ANOSIM (factors: farm effect and lease area). Lease area was included as a blocking factor to remove the substantial between lease variation.

Faunas associated with the three levels of farming treatment (reference, compliance and farm) were found to differ significantly from each other ($P<0.001$; Table 8), while faunas also differed significantly ($P<0.001$) between different farm lease areas. Farming effects were also evident within each of the D'Entrecasteaux Channel, Huon and Tasman Peninsula regions in individual regional analyses; however, no significant differences were found between farm, compliance and reference areas at Macquarie Harbour. The benthic faunas collected at Tasman Peninsula did not differ significantly between the lease areas studied.

Table 8. Significance (P) of rho-statistic calculated using two-way ANOSIM (factors: 'lease' area and farm 'effect') for farm leases grouped by geographical region.

Region	Farm effect		Lease area	
	Rho	P	Rho	P
All regions	0.52	<0.001	0.83	<0.001
D'Entrecasteaux Channel	0.41	0.014	0.35	<0.001
Huon	0.70	<0.001	0.79	<0.001
Tasman Peninsula	0.75	0.046	0.01	0.470
Macquarie Harbour	0.10	0.370	0.71	0.003

Faunal relationships were further examined using CAP analysis and data for all farm lease sites, where axes were calculated to maximise the separation of sites affected by the three farm impact levels. In the plot of the first two CAP axes (Fig. 4), samples from the farm treatment largely separate at the top right of the plot, samples from the compliance treatment in the bottom centre, and samples from the reference treatment at the top left. Faunal differences between the three impact treatments were found using the Trace statistic calculated by CAP to be significantly different ($p<0.001$).

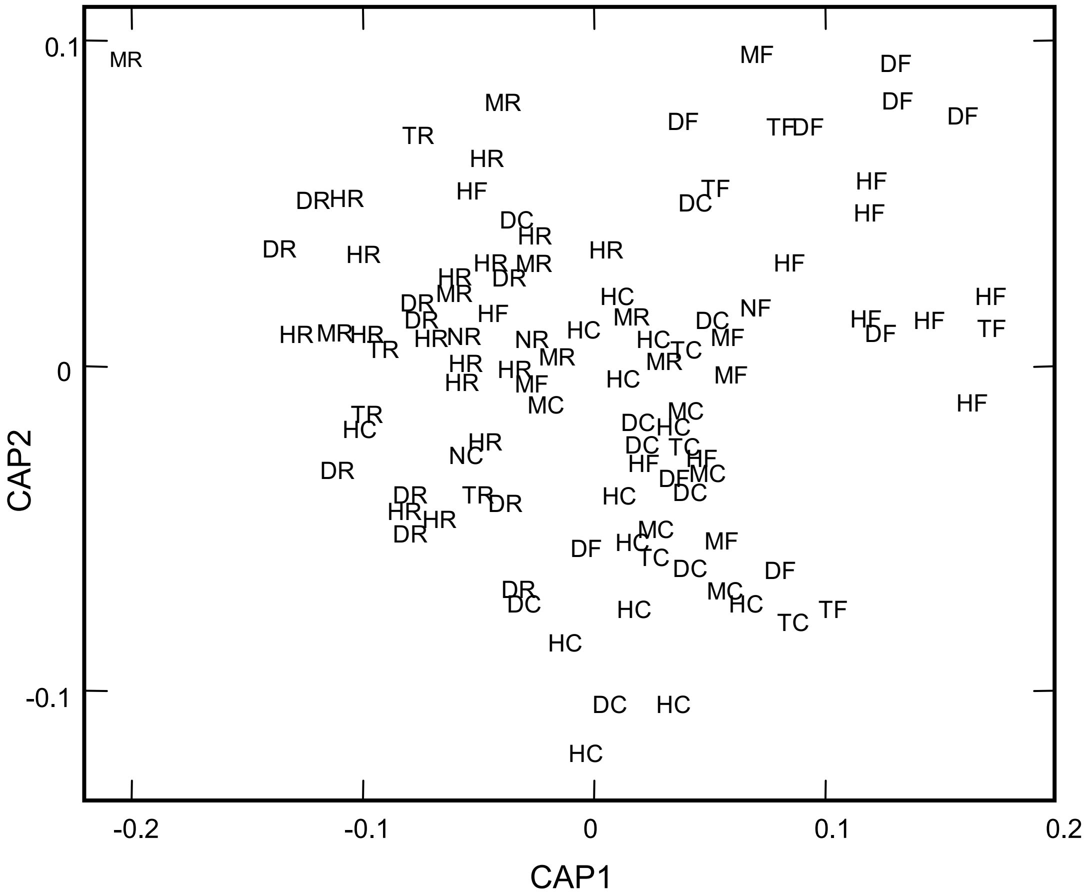

Figure 4. Macrofaunal relationships between different regions and levels of farm impact, as indicated by the first two principal components calculated by CAP analysis, with farm impact as grouping variable. The first letter in symbol refers to region (H: Huon; D: D'Entrecasteaux Channel; T: Tasman Peninsula; E: East coast; N: North coast; M: Macquarie Harbour), the second to farm impact level (R: reference; C: compliance; F: farm).

The CAP 'leave-one-out allocation of observations to groups' procedure (Table 9) indicated that the slight majority of reference sites (23 of 41) possessed faunas that would be correctly identified as typifying reference conditions, while the majority of farm sites (16 of 30) would be categorised as belonging to the farm impact group; however, the majority of compliance sites were misattributed to either reference or farm groups.

Table 9. Results of CAP 'leave-one-out allocation of observations to groups' for three farm impact groups.

Known	Allocated groups			Total	% correct
	Reference	Compliance	Farm lease		
Reference	23	15	3	41	56.1
Compliance	10	14	8	32	43.8
Farm	4	10	16	30	53.3

Species strongly associated with fish farming impacts were identified by calculating Pearson correlation coefficients that relate species abundance with principal CAP axes (Table 10). In relation to Fig. 4, species with strong positive correlations with CAP axes 1 and 2 are indicators of farm sites, species with strong negative correlations with CAP axis 2 are indicators of compliance sites, and species with strong negative correlations with CAP axis 1 and positive correlations with CAP axis 2 are indicators of reference sites.

Table 10. Species showing strongest Pearson correlations with CAP axes 1 and 2, with magnitude and direction of correlations.

Species	CAP1	CAP2	Indicator
Capitella sp.	0.571	0.223	Farm
Echinocardium cordatum	0.409	-0.057	
Glycera sp.	0.377	-0.090	
Ostracod sp.1	0.364	-0.121	Compliance
Nebalia sp.	0.358	0.115	Farm
Euphilomedes sp.	0.342	-0.139	Compliance
Theora lubrica	0.334	-0.065	
Chaetozone setosa	0.323	-0.169	Compliance
Neanthes cricognatha	0.323	0.181	Farm
Oedicerotid sp.	0.320	-0.032	
Ostracod sp.2	0.316	-0.186	Compliance
Mysella donaciformis	0.312	-0.150	Compliance
Nereid sp.	0.305	-0.101	Compliance
Halicarcinus rostratus	0.300	-0.018	
Euphilomedes sp.	0.285	-0.182	Compliance
Dimorphostylis cottoni	0.204	-0.235	Compliance
Tellina margaritina	0.151	-0.26	Compliance
Phyllodoce sp.	0.134	-0.289	Compliance
Philine angasi	0.112	-0.355	Compliance
Goniadid sp.	0.033	-0.378	Compliance
Apseudes sp.	0.028	-0.313	Compliance
Leptochelia ?dubia	-0.001	-0.336	Compliance
Asychis sp.	-0.055	-0.341	Compliance
Cirratulid sp.	-0.066	-0.329	Compliance
Callianassa limosa	-0.078	-0.299	Compliance
Dorvillea sp.	0.197	-0.224	Compliance
Polydora sp.	0.178	-0.238	Compliance

Several species (most notably *Capitella* sp., *Nebalia* sp. and *Neanthes cricognatha*) were strongly indicative of farm sites, while others (notably *Euphilomedes* sp. and *Chaetozone setosa*) were indicative of compliance sites; however, many species possessed high positive correlations with CAP axis 1 and little correlation with CAP axis 2 (e.g. *Echinocardium cordatum)*, hence were associated with both farm and compliance sites. Because faunas at reference sites varied greatly between locations, no species was identified as strongly indicative of reference conditions.

SIMPER was also used to identify species that differ greatly in density between farm impact levels. Based on the same data set as used for the ANOSIM analysis (Table 11), the most important indicators of farm impact were the polychaete *Capitella* sp., the introduced bivalves *Corbula gibba* and *Theora lubrica*, the ostracod *Euphilomedes* sp. and the gastropod *Nassarius nigellus* (Table 11). These same species were also the primary indicators of differences between farm and compliance sites. *Corbula gibba, T. lubrica* and *Euphilomedes* sp. were indicators of compliance sites when compared to reference sites, while the bivalve *Ennucula obliqua* and ophiuroid *Amphiura elandiformis* were more typical of reference sites than compliance sites.

Many of the same species identified in the Tasmanian-wide analysis were found to show consistent responses within different regions (Tables 12-14). In particular, the ostracod *Euphilomedes* sp. ranked as one of the top three indicators of both farm and compliance effects in all three major regions examined. Indicator species for Macquarie Harbour were not assessed because farming did not have any detectable impact on the benthos in that region (Table 8). At Macquarie Harbour, *Euphilomedes* sp. was the most abundant species in all three treatments (mean of 2 per sample), the reason for an elevated statewide mean for this species at reference sites in Table 11.

Table 11. Mean densities per sample for indicator species of farming effects across all Tasmanian regions. Indicator species were identified by SIMPER analysis for 28 sites across five regions, with rank in importance for paired comparisons between reference (R), compliance (C), and farm sites (F) shown in right columns.

Species	Reference	Compliance	Farm	R vs F	R vs C	F vs C
Capitella sp.	0.01	1.67	19.95	1		1
Corbula gibba	5.42	7.63	4.15	2	1	2
Theora lubrica	2.26	2.36	4	3	2	4
Euphilomedes sp.	1.05	2.22	4.09	4	3	3
Nassarius nigellus	1	1.36	2.63	5	10	5
Ampelisca cf. australis	1.81	1.56	1.63	6	7	8
Ennucula oblique	2.1	1.36	0.74	7	4	14
Amphiura elandiformis	1.57	1.19	0.45	8	5	
Thyasira sp.	1.03	0.1	0.11	9	8	
Leitoscoloplos bifurcatus	0.36	0.59	0.76	10	14	9
Heteromastus sp.	0.26	1.17	1.68	11		7
Maoricolpus roseus	1.22	1.45	0.38	12	6	12
Amphicteis sp.	0.55	0.69	0.92	13		
Kalliapseudes sp.	1.15	0.77	0.6	14	15	
Lumbrinereis sp.	0.98	1.38	0.69	15	9	13
Sphaeromatid sp.	0.21	2.81	1.42		11	6
Callianassa limosa	0.87	0.97	0.52		12	15
Asychis sp.	1.08	0.67	0.42		13	
Ostracod sp.1	0.38	0.99	1.42			10
Polydora sp.	0.26	0.58	0.83			11

Table 12. Mean densities per sample for indicator species of farming effects in the Huon region. Indicator species were identified by SIMPER analysis, with rank in importance for paired comparisons between reference (R), compliance (C), and farm sites (F) shown in right columns.

Species	Reference	Compliance	Farm	R vs F	R vs C	F vs C
Corbula gibba	5.77	6.87	7.26	1	1	2
Capitella sp.	0	0.15	8.97	2		1
Euphilomedes sp.	0.19	1	1.51	3	2	5
Terebellides sp.	0.51	1.09	1.01	4	3	
Amphiura elandiformis	2.37	1.69	0.6	5	15	9
Ennucula obliqua	2.92	1.34	0.77	6	4	10
Theora lubrica	1.43	2.56	3.36	7	11	6
Paraprionospio coora	0.71	0.82	1.34	8		3
Echinocardium cordatum	0.28	0.12	0.87	9	12	
Ostracod sp.	0.37	0.7	1.1	10	8	
Nemocardium thetidis	0.78	0.6	0.55	11	9	
Thyasira adelaideana	1.42	0.54	0.16	12	7	
Callianassa limosa	1.39	1.89	1.16	13		14
Asychis sp.	0.41	1.29	0.49	14	6	8
Sthenelais pettibonae	0.58	0.34	0.3	15		
Maoricolpus roseus	0.98	0.93	0.32		5	4
Lysilla jennacubinae	0.47	0.5	0.32		10	13
Kalliapseudes sp.	0.22	0.56	0.07		13	11
Amaena trilobata	0.43	0.33	0.08		14	
Amphicteis sp.	0.59	0.74	1.21			7
Euchone limnicola	0.15	0.5	0.48			12
Nebalia sp.	0	0.13	0.85			15

Table 13. Mean densities per sample for indicator species of farming effects for the D'Entrecasteaux Channel region. Indicator species were identified by SIMPER analysis, with rank in importance for paired comparisons between reference (R), compliance (C), and farm sites (F) shown in right columns.

Species	Reference	Compliance	Farm	R vs F	R vs C	F vs C
Capitella sp.	0.03	3.87	57.69	1		1
Euphilomedes sp.	0.27	2.57	5.59	2	2	3
Corbula gibba	11.68	18.09	5.3	3	1	2
Kalliapseudes sp.	3.44	1.91	1.95	4		8
Amphiura elandiformis	2.38	2.01	0.83	5	8	5
Nebalia sp.	0.06	0.2	5.6	6		4
Ennucula obliqua	3.65	3.05	1.64	7	6	7
Theora lubrica	5.51	4.32	9.01	8	7	
Nassarius nigellus	0.67	1.32	1.99	9	14	12
Ostracod sp.1	0.82	2.33	3.33	10	5	6
Byblis mildura	2.24	1.37	0.84	11	3	15
Ampelisca cf. australis	0.85	0.84	1.5	12		13
Nephtys australiensis	0.53	0.65	0.91	13		14
Lysilla jennacubinae	1.39	2.06	1.06	14	9	9
Maoricolpus roseus	1.07	1.18	0.33	15	4	10
Nemocardium thetidis	0.74	0.55	0.41		10	
Mediomastus australiensis	0.73	0.85	0.16		11	
Paraprionospio coora	0.52	0.58	0.55		12	
Amaena trilobata	0.51	0.67	0.35		13	
Ampelisca sp.	1.54	0.42	0.51		15	
Nassarius pyrrhus	0.09	1.1	1.59			11

Table 14. Mean densities per sample for indicator species of farming effects in the Tasman Peninsula region. Indicator species were identified by SIMPER analysis, with rank in importance for paired comparisons between reference (R), compliance (C), and farm sites (F) shown in right columns.

Species	Reference	Compliance	Farm	R vs F	R vs C	F vs C
Euphilomedes sp.	1.26	6.95	11.64	1	1	2
Ampelisca cf. australis	9.22	7.01	6.13	2	4	11
Pista australis	4.11	1.97	0.08	3	14	6
Asychis sp.	6.28	0.57	0.13	4	2	
Ostracod sp.2	0.03	0.95	1.27	5	9	
Mysella donaciformis	0.02	0.62	1.2	6	10	
Apseudid sp.	5.9	10.14	6.26	7	5	1
Terebellid sp.	3.31	0.77	2.1	8	8	9
Tipimegus thalerus	1.12	2	3.7	9	15	7
Nephtys australiensis	3.25	1.5	2.4	10	7	13
Ebalia intermedia	0.28	0.31	0.73	11		
Cyclaspis caprella	0.12	1.29	2.85	12		
Dimorphostylis cottoni	0.24	1.21	1.74	13		
Parawaldeckia stebbingi	0.22	1.74	2.11	14	11	12
Chaetozone setosa	0	0.39	0.96	15		
Maoricolpus roseus	3.95	5.34	1.19		3	10
Capitella sp.	0	3.57	1.83		6	4
Polydora sp.	0.25	2.11	4.97		12	3
Oweniid sp.	1.04	1.09	0.61		13	
Heteromastus sp.	0.55	6.12	10.05			5
Isaeid sp.	0.63	1.95	1.1			8
Nassarius pyrrhus	0.06	0.28	0.99			14
Tethygeneia sp.	1.23	1.26	2.73			15

Biennial change

Results of ANOVAs involving change in metrics for each two-year monitoring period (final less initial abundance) for sites that were initially reference sites and that were sampled on at least two occasions are shown in Table 15. As with other ANOVAs, where more than one site within a particular treatment within a farm lease was sampled, a mean was calculated and used in analyses. These ANOVAs were unbalanced because only 5 farm leases included reference sites that changed to farm sites, compared to 6 leases with reference sites that changed to compliance sites, and 25 farm leases with reference sites maintained as reference sites. For some metrics, the number of farm leases was reduced because of missing values (see degrees of freedom column in Table 15). Redox levels at 4 cm significantly declined by a mean of 134 eV at reference sites impacted by farming, but showed negligible change at reference sites converted to compliance sites (Fig. 5).

Table 15. Mean-square values (MS) and F-ratios resulting from one-way ANOVAs with farm as a fixed factor for 12 metrics of farming activity using data on change over two year monitoring cycles. * 0.01<P<0.05.

Metrics	df	MS		F
		Farm effect	Error	
Particle size	2/29	0.189	0.322	0.59
Silt-clay	2/29	31.4	65.3	0.48
Redox 0 cm	2/28	30200	10300	2.93
Redox 4 cm	2/28	27200	6000	4.53*
Organic matter	2/29	1.84	4.58	0.40
Total species	2/33	80.3	168.0	0.48
Total abundance	2/33	14000	16500	0.85
Dominance	2/33	0.007	0.029	0.25
Simpson's Index	2/33	5.4	13.6	0.40
Introduced species	2/33	91	397	0.23
Capitellids	2/33	46.3	28.9	1.60
Bivalves/molluscs	2/31	187	545	0.34

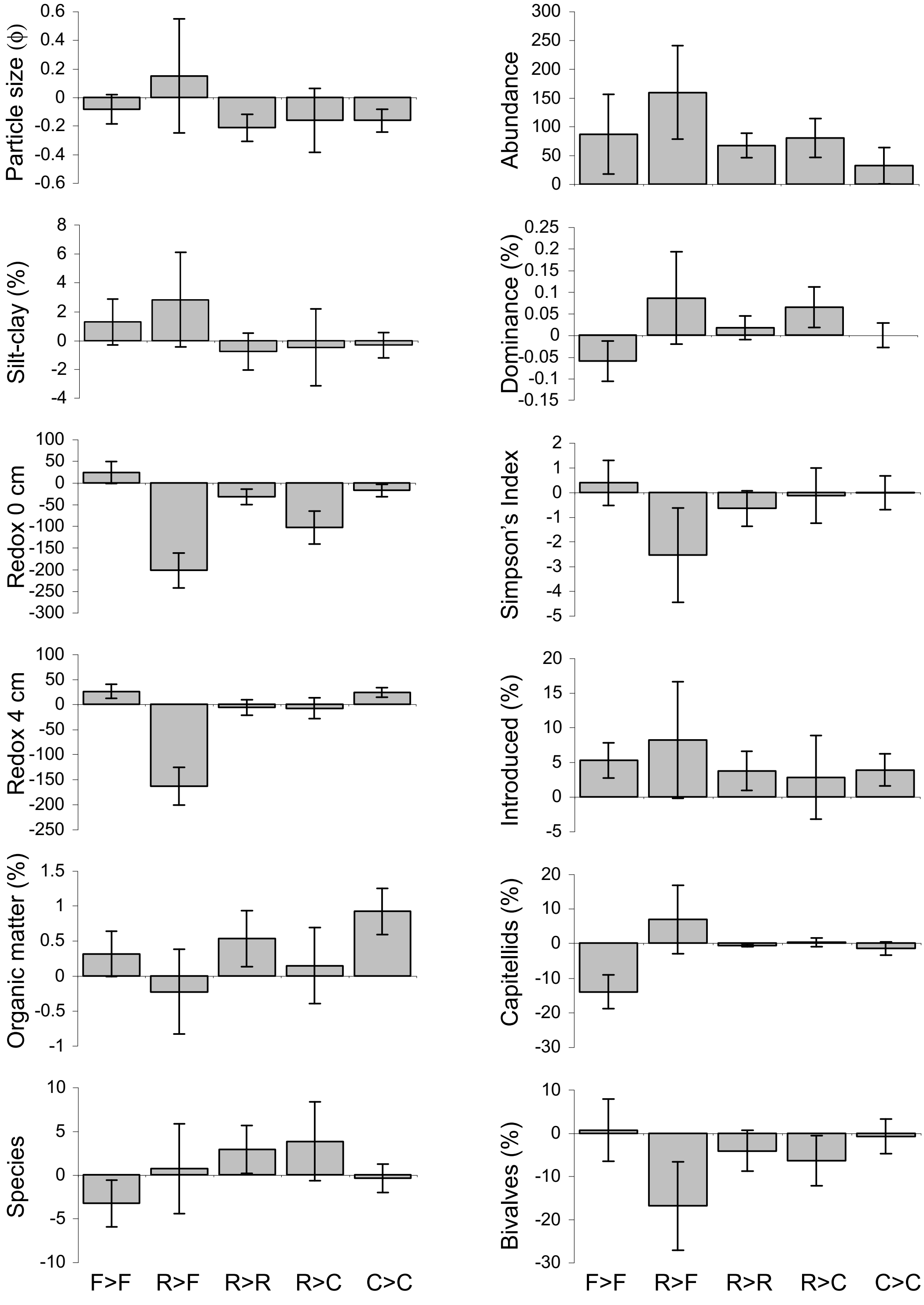

Figure 5. Mean change (±SE) per two year monitoring cycle in 12 metrics of farm activity for farm sites maintained as farm sites (F>F), reference sites converted to farm sites (R>F), reference sites maintained as reference sites (R>R), reference sites converted to compliance sites (R>C), and compliance sites maintained as compliance sites (C>C).

Although ANOVAs indicated that only redox at 4 cm depth significantly responded to farm activity between start and end of the biennial monitoring cycle (Table 15), other metrics previously implicated as affected by farming (see Table 6) trended in the direction predicted for fish farm effects and generally exhibited substantial change. At sites converted from reference to farming, redox potential at the sediment surface declined by an average of 109 eV, the bivalve:mollusc ratio declined by 11%, the percentage of capitellid polychaetes in the fauna increased by 4%, and proportional dominance of the most abundant species increased by 7 %. These four metrics, and redox potential at 4 cm, all exhibited negligible change at reference sites maintained as reference sites and compliance sites maintained as compliance sites (Fig. 5). The proportion of capitellids in samples at farm sites maintained as farm sites declined through the biennial sampling cycle.

Two univariate metrics of farm activity – redox potential at the sediment surface and total macrofaunal density – changed significantly through time independently of farm treatment, as indicated by the overall change during two-yearly monitoring periods differing significantly from 0 in two-tailed T-tests using mean data per farm for reference sites maintained as reference sites (Table 16).

If the power of tests of background change through time is increased by adding compliance sites maintained as compliance sites to this analysis, then six metrics show significant change through time (Table 16). Organic matter, animal abundance, modal particle size and proportion of introduced taxa increased significantly during the monitoring program, while sediment surface redox and proportional abundance of capitellids decreased.

Table 16. Percentage change over two-year monitoring cycle at reference sites sampled on more than one occasion, with result of T-test where significance of difference between mean change and no change is assessed (n=25). Similar information is presented for analyses involving data for compliance sites maintained as compliance sites in addition to reference sites maintained as reference sites (n = 46).

Metrics	Reference sites			Reference sites + compliance sites		
	Change	SE change	t	Change	SE change	t
Particle size (phi)	-0.15	0.12	-1.24	-0.17	0.08	-2.33*
Silt-clay	-0.65	1.66	-0.39	-0.48	0.97	-0.49
Redox 0 cm	-43.02	20.25	-2.13*	-29.35	12.83	-2.29*
Redox 4 cm	-8.97	14.69	-0.61	12.35	10.69	1.16
Organic matter	0.67	0.53	1.26	0.91	0.34	2.68*
Total species	3.58	2.43	1.47	1.39	1.49	0.94
Total abundance	77.25	26.03	2.97**	60.56	17.74	3.41***
Dominance	0.04	0.03	1.17	0.03	0.02	1.31
Simpson’s Index	-0.88	0.72	-1.21	-0.6	0.48	-1.25
Introduced species	5.37	3.75	1.43	7.08	2.71	2.61*
Capitellids	-0.71	0.55	-1.28	-0.89	0.40	-2.22*
Bivalves/molluscs	-5.62	5.17	-1.09	-3.84	3.38	-1.14

Analyses involving change in density of widespread species over the two-year monitoring cycle indicated that a number of species were significantly affected by farm impact (ANOVA, Table 17) and time (T-test, reference site data, Table 17). Three of the four most important species identified in spatial analyse as affected by farm impact (*Capitella* sp., *Theora lubrica* and *Euphilomedes* sp.) were significantly more abundant at reference sites converted to farm sites, while the tanaid *Kalliapseudes* sp. showed a significant density decline (Table 17; Fig. 6). Five species (*Corbula gibba, Ennucula obliqua, Leitoscoloplos bifurcatus, Nemocardium thetidis* and *Lysilla jennacubinae*) significantly increased in abundance at reference and compliance sites over the biennial monitoring cycle (T-test in Table 17; Fig. 6).

Table 17. Results of one-way ANOVAs (df=2/32) to assess whether change over two-year monitoring cycle differs between farm impact treatments. Results of t-tests to assess change through time (i.e. whether mean change significantly differs from 0) at reference sites maintained as reference sites is also given (n=25). **, 0.001<P<0.01; * 0.01<P<0.05.

Species	ANOVA			T-test
	Farm impact	Error	F	t
Capitella sp.	2.83	0.49	5.81**	1.00
Corbula gibba	0.70	2.74	0.26	2.68*
Theora lubrica	4.98	1.37	3.63*	1.52
Euphilomedes sp.	2.36	0.66	3.58*	1.57
Nassarius nigellus	1.80	1.46	1.23	1.2
Ampelisca cf. australis	0.23	0.99	0.24	1.91
Amphiura elandiformis	0.73	1.02	0.72	1.49
Ennucula obliqua	2.34	1.09	2.16	2.39*
Thyasira sp.	0.12	0.48	0.26	0.77
Leitoscoloplos bifurcatus	0.86	0.50	1.73	2.99**
Heteromastus sp.	0.19	0.54	0.36	2.06
Maoricolpus roseus	0.45	1.54	0.29	1.19
Callianassa limosa	1.48	0.93	1.59	1.53
Ostracod sp.1	1.32	1.05	1.25	0.91
Kalliapseudes sp.	5.33	1.38	3.86*	0.93
Nemocardium thetidis	0.32	0.86	0.37	2.66*
Nemertean sp.	0.18	0.44	0.42	1.81
Lumbrinereis sp.	1.65	1.41	1.17	0.54
Chaetozone setosa	0.31	0.66	0.47	0.39
Lysilla jennacubinae	0.10	0.91	0.12	3.30**

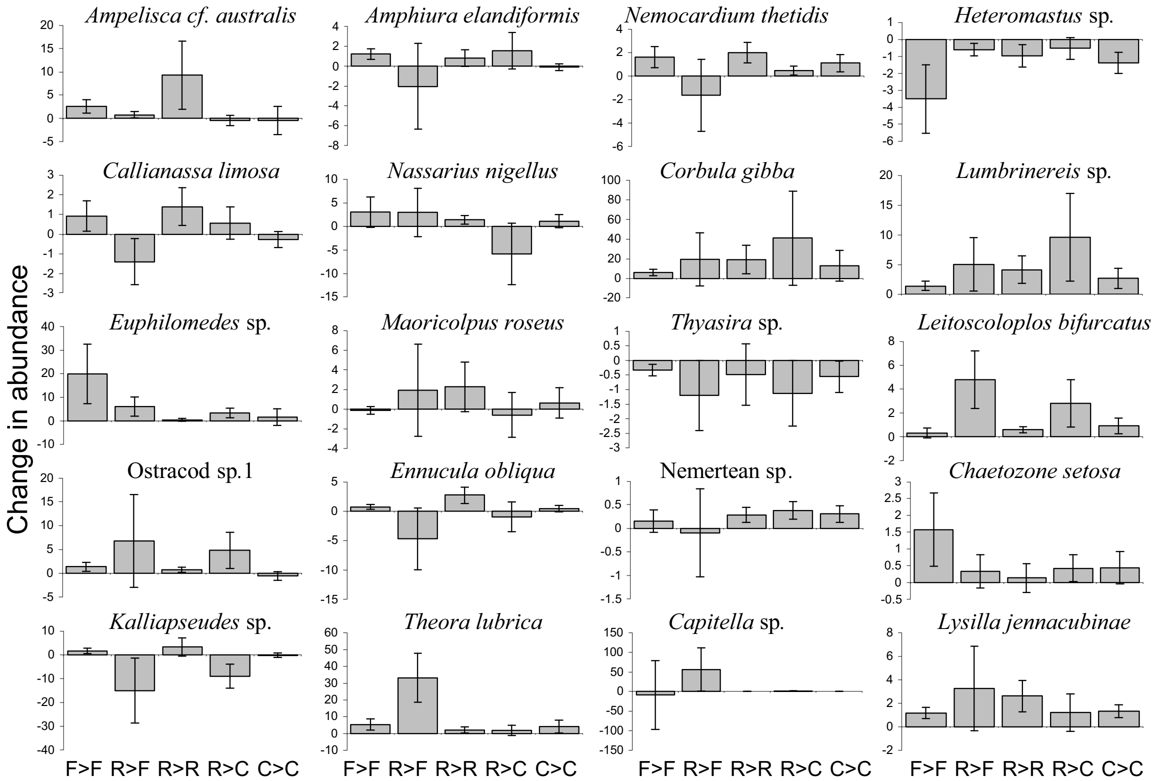

Figure 6. Mean change (±SE) per two year monitoring cycle in the abundance of the 20 most widespread species for farm sites maintained as farm sites (F>F), reference sites converted to farm sites (R>F), reference sites maintained as reference sites (R>R), reference sites converted to compliance sites (R>C), and compliance sites maintained as compliance sites (C>C).

3.4 Marine pest associations and impacts

Overall, a total of 10 introduced and 4 cryptogenic species were identified during surveys (Table 4). Introduced taxa were abundant in all regions other than Macquarie Harbour, where the only introduced animal collected was a single specimen of *Maoricolpus roseus*. Three introduced mollusc species (*Corbula gibba, Theora lubrica* and *M. roseus*) occurred in sufficiently high abundance to warrant individual investigation of associations and impacts.

Table 18. Abundance and site occurrence for introduced and cryptogenic (cosmopolitan and possibly introduced) species recorded during sampling. Each site included three replicate grab samples, with species recorded at the same location on two sampling occasions tabulated here as occurring at two sites.

Species	Number	Sites	Taxon
Introduced taxa			
Corbula gibba	8831	248	Mollusc bivalve
Theora lubrica	3887	236	Mollusc bivalve
Maoricolpus roseus	1878	174	Mollusc gastropod
Euchone limnicola	596	103	Polychaete
Caprella acanthogaster	172	50	Crustacean
Raeta pulchella	97	31	Mollusc bivalve
Petrolisthes elongatus	21	14	Crustacean
Cancer novaezealandiae	4	3	Crustacean
Asterias amurensis	4	3	Echinoderm
Musculista senhousia	2	2	Mollusc bivalve
Halicarcinus innominatus	1	1	Crustacean
Cryptogenic taxa			
Leptochelia dubia	153	49	Crustacean
Amphipholis squamata	57	18	Echinoderm
Mytilus galloprovincialis	26	12	Mollusc bivalve
Caprella equilibra	13	6	Crustacean

The population of *Theora lubrica* increased over the period of study at farm and compliance sites but not at reference sites (Fig. 7), a significant interactive change when assessed by Analysis of Covariance (Table 19). *Corbula gibba* was not affected by farm effects; however, populations of this species increased significantly (Table 19), and by an order of magnitude (Fig. 7), across the region over the initial two-year monitoring cycle, and then declined during the following two year period. Densities of *Maoricolpus roseus* did not change significantly with time nor exhibit a significant relationship with farm effect (Fig. 7; Table 19).

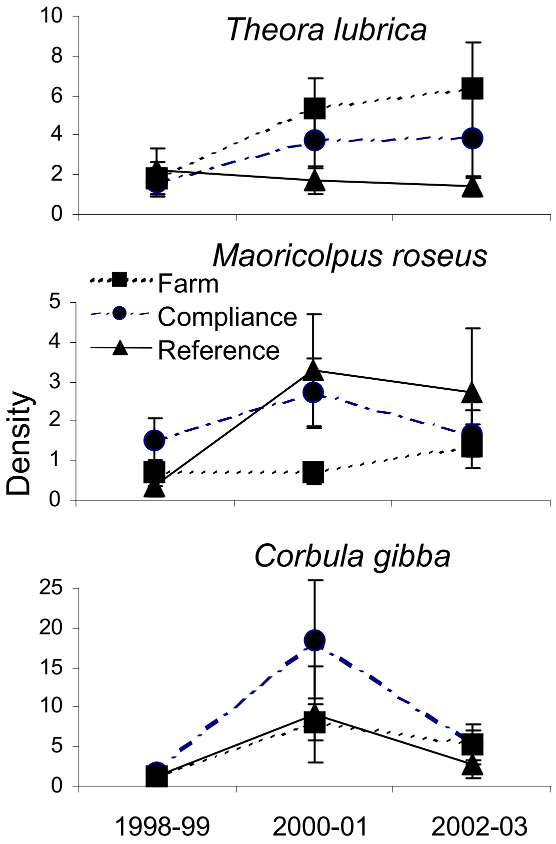

Figure. 7. Mean abundance per sample (±SE) of three introduced species at sites affected by different farm treatments over three biennial monitoring cycles.

Table 19. Mean-square values (MS) and F-ratios resulting from Analysis of Covariance (fixed factor: farm effect) for abundance of three introduced species in three different time periods (1998-99, 2000-01, 2002-03). Mean data were used for each farm effect level at each farm lease, with farm lease included in the model as a blocking factor, which was significant in all three cases (df = 26/129, F>5.5, p<0.001). Degrees of freedom are 1/129, 2/129 and 2/129 for year, farm effect, and year x farm effect, respectively. ***, P<0.001; * 0.01<P<0.05.

Species	Year		Farm effect		Year x Farm effect		Error
	MS	F	MS	F	MS	F	MS
Theora lubrica	5.647	14.17***	1.742	4.37*	1.743	4.38*	0.398
Maoricolpus roseus	0.267	0.69	0.255	0.66	0.254	0.66	0.386
Corbula gibba	12.60	24.54***	0.320	0.62	0.320	0.62	0.513

Associations between the three predominant introduced species and environmental conditions were assessed by calculating Spearman rank correlations between change in density of the species over the first biennial monitoring period at sites sampled on more than one occasion and environmental metrics at the time of initial sampling. Data from Macquarie Harbour were excluded from this analysis because of the negligible occurrence of introduced taxa in that region. *Corbula gibba* and *Theora lubrica* were both significantly associated with sediment characteristics, exhibiting strongest rises in population densities at sites with fine particles and high organic content (Table 20). *Maoricolpus roseus* did not respond to any of the monitored environmental metrics.

Table 20. Spearman rank correlations (r_s) relating change in population density over biennial monitoring cycle of three introduced species with environmental metrics at commencement of monitoring cycle (n=75). ***, P<0.001; **, 0.001<P<0.01; * 0.01<P<0.05.

Metric	*Theora*	*Maoricolpus*	*Corbula*
Depth	-0.106	-0.126	-0.113
Phi	0.250*	0.025	0.414***
Silt	0.253*	0.058	0.294*
Redox 0 cm	0.133	0.135	-0.182
Redox 4 cm	0.026	0.136	-0.144
Organic	0.285*	0.067	0.265*

The similarity of *T. lubrica* and *C. gibba* in their response to environmental factors was partly a consequence of the two species often showing large increases in abundance at the same sites. *Theora lubrica* and *C. gibba* were significantly correlated with each other when changes in population numbers of the three common introduced species over the biennial cycle were correlated with changes in numbers of the 100 most widespread species (Table 21). The strongest correlation in density change existed between *T. lubrica* and another introduced bivalve, *Raeta pulchella*. The strongest correlations between introduced species and other taxa were in all but one case positive, indicating that either the three main introduced taxa were responding to conditions also preferred by native taxa, or the introduced taxa had a beneficial effect on populations of native taxa. The observed exception was a negative correlation between *C. gibba* and the maldanid polychaete *Clymenella* sp., an indication that population growth of the introduced species may have negatively affected population numbers of *Clymenella* sp.

Table 21. Spearman rank correlations (r_s) relating change in population density over biennial monitoring cycle of three introduced species with change in density of associated species (n=75). Only correlations with P<0.01 are shown.

Species	r_s	P
Theora lubrica population trend correlations		
Nassarius pyrrhus	0.212	0.008
Corbula gibba	0.249	0.002
Thyasira adelaideana	0.281	<0.001
Nereid sp.	0.284	0.002
Raetta pulchella	0.309	<0.001
Corbula gibba population trend correlations		
Clymenella sp.	-0.207	0.009
Nephtys longipes	0.207	0.009
Hexapus granuliferus	0.213	0.007
Nemocardium thetidis	0.226	0.004
Theora lubrica	0.249	0.002
Sthenelais pettibonae	0.263	0.001
Maoricolpus roseus population trend correlations		
Ennucula obliqua	0.206	0.010
Anthurid sp.	0.220	0.006
Eupolymnia koorangia	0.240	0.002
Ebalia intermedia	0.280	<0.001

4. Discussion

4.1 Regional effects

A pronounced regional separation in the benthic macrofauna exists between sites at Macquarie Harbour and sites on the eastern and northern Tasmanian coasts. This basic subdivision of the inshore biota has been noted previously in a biogeographic study of Tasmanian estuaries, where sites along the southern and western Tasmanian coasts were recognised as possessing a highly depauperate and distinctive fauna compared to sites studied along the eastern and northern coasts (Edgar *et al.* 1999). The most likely reason for this ecological dichotomy is the combination of nutrient poor waters, poor soils, absence of significant anthropogenic nutrient loading to rivers and estuaries, and low light penetration through tannin-stained surface layers (Edgar *et al.* 1999). Together these factors cause low phytomicrobenthos and phytoplankton productivity across the western region, and generate a poor trophic base to estuarine food webs, including within Macquarie Harbour.

The benthic macrofauna in all regions of the state, including Macquarie Harbour, appears to be undergoing a period of rapid change. Two environmental metrics changed significantly with time in analyses involving reference sites maintained as reference sites, and six of the twelve environmental metrics produced significant results when compliance sites maintained as compliance sites were added to the data set analysed (Table 16).

Assumptions when combining data from compliance sites with reference sites are: (i) that any farm impacts at compliance sites are small and remain constant with time, and (ii) that the 1-2 km distance between most compliance sites and reference sites associated with the same farm was sufficient for these two sets of sites to be regarded as independent. The first assumption, that changes at compliance sites are not greatly influenced by farm impacts, is supported by similar mean values of change over the biennial cycle regardless of whether compliance sites are included in analyses (Table 16). Although the second assumption of independence of farm and compliance sites may not seem appropriate given that compliance sites were paired with reference sites, it is supported by the density of farm leases in regions studied and the fact that some reference sites were located approximately equidistant between two farm leases. In one case, a single reference site was used for two lease areas.

The difference in number of significant results between the two sets of tests clearly relates primarily to number of replicates and power of tests, rather than to effect size or a major difference between mean values at reference and compliance sites in trends through time. This outcome emphasises the generally low power of tests at regional scales and the value of the collaborative approach utilised here between researchers, industry and management, which allowed many more farm leases to be studied than in previous published investigations. Despite substantial changes in mean values through time, data from a total of 46 (cf. 25) independent locations were needed to generate significant results at a statewide scale for four important univariate metrics. Virtually all other studies of fish farm impacts have focused on less than five locations.

Environmental metrics that changed significantly over the period of study included higher organic loadings to sediments, reduced redox potential, and reduced phi value (increased modal particle size). The first two of these trends possibly reflect increasing human impact through increased organic loadings to the inshore marine environment. By contrast, increasing modal particle size is indicative of improving environmental conditions through either increased deposition of shell particles or winnowing of fine particles from the seabed.

Associated with the net environmental changes were changes in the abundance of many species, and an overall increase in the total density of macrofauna, proportional abundance of capitellids, and proportional abundance of introduced species. The majority of species exhibited trends for increase during the study period. Only 2 of the 20 individual species examined in detail exhibited declining population trends at reference sites (the bivalve *Thyasira* sp. and the polychaete *Heteromastus* sp.; Fig. 6), and in neither case was this decline significant (Table 17).

Increasing densities of native species should be regarded as a positive environmental trend because it contrasts with a prolonged period of population decline for inshore molluscs over the past century (Edgar, Samson, 2004). It is notable that two native bivalve species – *Nemocardium thetidis* and *Ennucula obliqua* – showed significantly increased population numbers over the period of study (Table 17). Recent population increases amongst the macrobenthos possibly reflect improving environmental conditions following more effective management of marine, estuarine and coastal terrestrial habitats.

The negative side of patterns of increasing macrofauna observed is that the abundance of introduced taxa is increasing more rapidly than the abundance of native species, with a significant proportional increase in abundance through time. Continued long-term monitoring of reference sites is needed to more accurately assess the spatial and temporal scale of changes, and to allow a rigorous assessment of whether the threat posed by increased introduced pest species exceeds benefits to biodiversity resulting from improved environmental conditions.

4.2 Fish farm impacts

Fish farm impacts that extend to regional scales and affect reference sites could not be assessed using the experimental design applied in this project, given that no reference regions without fish farms were investigated through time. Nevertheless, any regional impact of fish farming should be signalled by net change at reference sites. In this context it is notable that the percentage organic matter in sediments, redox potential of surface sediments, total macrofaunal abundance, proportional abundance of capitellid polychaetes, and proportional abundance of introduced species, all increased significantly through time at reference and compliance sites (Table 16). These trends are consistent with fish farms slightly enriching sediments with organic matter at regional scales. Given the environmental importance of any regional impacts, further investigation is urgently warranted through an extension of monitoring of reference sites to regions lacking salmonid farms (e.g. Port Davey, Great Oyster Bay, Mercury Passage, Furneaux Group). The magnitude of change through time at reference sites in farming regions relative to non-farming regions would then provide an index of farming effects at the regional scale.

Five univariate metrics showed significant impacts associated with farm leases and can be regarded as indicators of farming activity: (i) redox potential at the sediment surface, (ii) redox potential at 4 cm depth, (iii) the proportional abundance of capitellids, (iv) the bivalve/mollusc ratio, and (v) the proportional dominance of the most abundant species. These metrics all significantly differed between treatments in the spatial comparison (Table 6), and all trended in the appropriate direction when changes at reference sites converted to farm or compliance sites were assessed through time (Fig. 5). Nevertheless, despite relatively large changes through time, including a reduction in redox potential of 150 eV at 4 cm depth, the change through time at reference sites converted to farm sites was only found to be significant for redox potential at the sediment surface (Table 15). This low level of significance in temporal tests presumably reflected low statistical power, a consequence of data on changes from reference to farm conditions being available for only five lease areas, and large regional differences in faunas between farms. In contrast to spatial comparisons, regional effects could not be factored out of temporal tests because of low and inconsistent farm replication between regions. An additional factor contributing to low analytical power was the high level of variation at sites sampled within farm leases, which reflects patchiness in local distribution of impacts. This patchiness relates to variability in the distance that sites sampled were located from cages and the fallowing history of sites.

All five metrics that differed significantly between farm, compliance and reference sites showed greatest difference between reference and farm sites, with intermediate values at compliance sites (Fig. 3). Thus, effects of farming activity detectably extended to compliance sites but at diminished levels. Similar outcomes were evident in multivariate analyses of samples; the CAP 'leave-one-out allocation of observations to groups' indicated that some compliance sites overlapped with both reference sites and farm sites, while reference or farm sites that were misclassified were generally classed as compliance sites.

Similarly, species that were identified as the most important indicators of farm effects relative to reference sites also emerged in comparisons between compliance and reference sites, with average densities of these indicator species intermediate at compliance sites compared to farm and reference sites. For example, the polychaete *Capitella* sp. was an order of magnitude more abundant at farm sites than compliance sites but, while rarely present at control sites, still occurred in moderate abundances at compliance sites (Table 11).

Densities of *Capitella* sp. provided a more reliable indicator of farm impacts than the family level indicator 'percentage abundance of capitellids'. Percentage for all capitellids lacked a significant relationship with farm activity in the temporal analysis (Table 15), whereas the comparable test for *Capitella* sp. showed a highly significant result (Table 17). Different species of capitellid apparently responded in different ways to environmental conditions, particularly the two most abundant taxa *Capitella* sp. and *Heteromastus* sp. (see Fig. 6), diluting the signal of farming impacts when capitellid species were grouped within family.

In addition to *Capitella* sp., other important species indicators of farm impact were the introduced bivalve *Theora lubrica*, the ostracod *Euphilomedes* sp., and the gastropod *Nassarius nigellus* (Table 11), all of which generally increased in density at reference sites converted to farm sites (Fig. 6), and the crustacean *Nebalia* sp. and the nereid polychaete *Neanthes cricognatha* (Table 10), both of which were locally abundant. All of these species have been identified as fish farm indicators in previous studies (Edgar *et al.* 2005; Macleod, *et al.* 2004).

While a number of species were identified as strongly positively associated with fish farm impacts, species negatively affected by farm impacts were less evident. This outcome derives in part from most of the positive indicator species of farm impact being widely distributed around the eastern and northern Tasmanian coasts, presumably opportunistically tracking organically-enriched sediments such as associated with fish farms. By contrast, macrofauna present at undisturbed sites showed a higher degree of regional variation, hence consistent patterns of faunal decline did not emerge for individual species when analyses were undertaken on a statewide basis. Nevertheless, population densities of many regionally-restricted species showed major declines. This is most evident in the SIMPER analysis for the Tasman Peninsula (Table 14), the region with non-significant faunal variation between sites (Table 8), where three of the four strongest species indicators of farm impacts relative to reference sites were species with declining rather than increasing population numbers (*Ampelisca* cf. *australis, Pista australis* and *Asychis* sp.).

4.3 Introduced marine pest impacts

Introduced marine pests were abundant across all regions studied in Tasmania, other than at Macquarie Harbour, where only a single specimen of an introduced species, the New Zealand screwshell *Maoricolpus roseus*, was recorded. Approximately 15% of all benthos collected statewide comprised known introduced taxa (Fig. 3), with this percentage increasing by 2-3% per annum (Table 16; Fig. 5). These data extend results of the 1997-99 baseline study undertaken in the Huon, D'Entrecasteaux Channel and Tasman Peninsula regions, where 10% of total macrofaunal densities and 45% of total macrofaunal biomass was found to comprise introduced taxa (Edgar *et al.* 2005). Given increasing abundance since 1998 and the relatively large individual biomass of introduced taxa such as *M. roseus*, the percentage of total macrofaunal biomass composed of introduced species probably now exceeds 50% on average for habitats monitored across Tasmania. Given this scale of introductions, exotic species presumably comprise a major threat to biodiversity within the state.

We note also that estimates of the total density of introduced taxa provided here may be substantially lower than the true level because they do not include introduced species that are not currently recognised as such, including cryptogenic species and taxa not identified to species level during our study. Future research will likely show that some of the species identified to genus level in this project are in fact introduced, particularly amongst widely-distributed taxa that are opportunistically associated with organically-enriched sites (e.g. *Capitella* sp. and *Euphilomedes* sp.).

Amongst the most common introduced species, the bivalves *Theora lubrica* and *Corbula gibba* (and probably also *Raeta pulchella*, which was positively associated with *T. lubrica*) are associated with fine sediments with high organic loadings. By contrast, *Maoricolpus roseus* lacked a significant relationship with environmental metrics and exhibited stable population numbers throughout the study. The *C. gibba* population appears to be newly established within the Tasmanian region, as indicated by low densities at the commencement of the study, explosive growth between 1998 and 2001, and population decline over the 2002-03 biennial monitoring cycle.

Populations of *Theora lubrica* increased during the period of study, but only at fish farm sites and, to a lesser extent, compliance sites. Fish farms clearly provide favourable conditions for the growth of *T. lubrica* populations, probably because of increased deposition of fine organically-enriched particles in the vicinity.

Although *Corbula gibba* was implicated in SIMPER analysis as also exhibiting increased abundance at farm sites, density differences between farm, compliance and reference sites were low, and this bivalve showed a comparable change through time at reference sites converted to compliance sites as at reference sites maintained as reference sites. Thus, an association between *C. gibba* and fish farms is ambiguous and should be regarded as low or non-existent.

No substantive negative environmental impact of introduced species on native species was evident in this study. Increased abundance of an introduced species at a site was generally associated with no change or increased abundance of native species, such as increased numbers of the predatory polychaete *Sthenelais pettibonae* at sites where populations of *Corbula gibba* were increasing, and increased numbers of the predatory crab *Ebalia intermedia* at sites showing population increases of *Maoricolpus roseus* (Table 21). The exception to this pattern was an observed decline in the abundance of the maldanid polychaete *Clymenella* sp. at sites with increased abundance of *C. gibba*. This result warrants further investigation as it may be real or reflect a Type I statistical error, where an association is incorrectly identified as significant because of the large number of species tested in this analysis.

Given the ubiquity and rapidly increasing abundance of introduced taxa, much stronger tests of interactions between introduced and native taxa are needed as a matter of urgency. If introduced taxa negatively affect native species with highly localised distributions, then such interactions would not have been detected with the level of statistical power in the statewide analyses undertaken here. Manipulative investigations using appropriate experimental designs are required to properly assess impacts of the most common introduced taxa, including the investigation of changes through time in native faunas in plots and sediment trays that are treated with additions and removals of introduced taxa.

Overall, the Tasmanian salmonid farm monitoring program has provided an invaluable state-wide baseline for assessing environmental impacts within estuarine and inshore marine habitats, and will provide a benchmark for future monitoring and manipulative studies through the future. It represents a highly successful collaboration between researchers, industry and management. Given the magnitude of ecological changes evident over the six-year period of study, the Tasmanian finfish monitoring scheme should be recognised as a critical resource for informing management action, and expanded through the long-term, particularly with the inclusion of reference sites within regions unaffected by fish farms.

5. Acknowledgements

Field sampling was undertaken or supported by Aquenal Pty Ltd, Tassal Operations Ltd, Huon Aquaculture Company Pty Ltd, AMD Pty Ltd, Dom O.Brien and Aquatas Pty Ltd. Laboratory analysis and data compilation were undertaken by Aquenal Pty Ltd. Commitment and support to the project by Ron Mawbey, Derek Shields, Karen Parsons, Kathryn Redburn, Sasha Migus, Ed Forbes, Kate Hoyle (Aquenal), Trevor Dix (Tassal), and Christine Crawford (TAFI) made a state-wide study with rigorous and consistent methodology possible. Comments on the draft manuscript by Catriona Macleod were much appreciated. The map was provided by Jeremy Dudding.

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7. Appendix 1. Data (on CD)

The data recorded in this study can be viewed on the attached CD. This includes three tables with information of sites, metrics and dates sampled as well as the species recorded and their abundance per site.